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Fructose-induced aberration of metabolism in familial gout identified by ^{31}P magnetic resonance spectroscopy

(arthritis/metabolic disease/inborn error of metabolism/defect in fructose metabolism/noninvasive assessment of metabolism)

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ABSTRACT **The hyperuricemia responsible for the development of gouty arthritis results from a wide range of environmental factors and underlying genetically determined aberrations of metabolism. ^{31}P magnetic resonance spectroscopy studies of children with hereditary fructose intolerance revealed a readily detectable rise in phosphomonoesters with a marked fall in inorganic phosphate in their liver *in vivo* and a rise in serum urate in response to very low doses of oral fructose. Parents and some family members heterozygous for this enzyme deficiency showed a similar pattern when given a substantially larger dose of fructose. Three of the nine heterozygotes thus identified also had clinical gout, suggesting the possibility of this defect being a fairly common cause of gout. In the present study this same noninvasive technology was used to identify the same spectral pattern in 2 of the 11 families studied with hereditary gout. In one family, the index patient's three brothers and his mother all showed the fructose-induced abnormality of metabolism, in agreement with the maternal inheritance of the gout in this family group. The test dose of fructose used produced a significantly larger increment in the concentration of serum urate in the patients showing the changes in ^{31}P magnetic resonance spectra than in the other patients with familial gout or in nonaffected members, thus suggesting a simpler method for initial screening for the defect.**

Environmental factors and underlying genetic defects of metabolism can contribute to the hyperuricemia required for the deposition of monosodium urate crystals in and about the joints, which is characteristic of gouty arthritis (1). Among the environmental factors are such widely disparate causes as lead poisoning, alcohol ingestion, starvation, and obesity. The genetic heterogeneity of the disease is shown by the range of primary defects in metabolism of purines, carbohydrates, and amino acids identified as causes of hyperuricemia through impairment of urate excretion by the kidney, by increasing the rate of urate synthesis in the body, or by a combination of these mechanisms (2). Dietary approaches to the control of the hyperuricemia and gout by limitation of the intake of purines and of alcoholic drinks initiated in the previous century were of limited value, but the introduction of uricosuric drugs and subsequently of allopurinol has made possible the very effective control of the hyperuricemia and the clinical features of the disease in a preventive mode by primary care physicians. Although a substantial number of specific enzyme abnormalities have now been identified as primary causes of gouty arthritis, most of them are very rare disorders responsible for overproduction of uric acid and the resultant more severe manifestations of gout (1). Relatively little progress has been made in the identification of the types of metabolic defects responsible for the more common dominantly inherited familial gout seen by practicing physicians.

Hereditary fructose intolerance is known to be associated with hyperuricemia although the major clinical manifestations, as the name implies, have to do with severe or even fatal adverse reactions to ingested fructose as a result of a recessively inherited defect of aldolase B, resulting in loss of its capacity to split fructose 1-phosphate (3). A publication (4) has claimed the putative identification of the abnormal gene for fructose intolerance. By using conventional biochemical tests, only intestinal biopsy has provided a method for the detection of heterozygotes (5). However, heterozygotes were readily identified noninvasively in studies of affected and unaffected family members by using *in vivo* ^{31}P magnetic resonance (MR) spectroscopy of the liver before and after an oral fructose load (6). The homozygous affected children showed a marked increase in the phosphomonoester (PME) peak and a substantial decrease in the peak for inorganic phosphate (P_i) in response to very low doses of oral fructose. The parents, as expected for obligate heterozygotes, along with some of the siblings and other clinically unaffected family members showed a similar pattern in response to much larger doses of oral fructose (6). Of special interest was the finding of clinical gout in three of the nine heterozygotes identified by MR spectroscopy (6). The calculated incidence of the heterozygote in the European population [1/80 in Switzerland (3) to 1/250 in Great Britain (6)] is of sufficient frequency to account for a significant portion of gout patients. Infusion of fructose increases serum urate in normal children, in children with hereditary fructose intolerance (7), and in gouty and nongouty adult volunteers (7, 8).

In the present study, MR spectroscopy was used to monitor the changes induced by a 50-g load of oral fructose in the P_i and PME peaks in the liver of a group of 11 volunteers with familial gout. In addition, an indication of the magnitude of endogenous uric acid production of each patient was obtained and the effect of the fructose load on serum urate was followed. In one index patients showing MR spectroscopic evidence of the metabolic aberration of fructose metabolism, the effect of a fructose-restricted diet on the hyperuricemia was also assessed. In the other index patient, the early affect of the low-fructose diet on uric acid production was measured in the urine for 24 hr.

EXPERIMENTAL PROCEDURES

Patients. Patients were selected from among gout patients referred by primary care physicians and rheumatologists of

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Abbreviations: MR, magnetic resonance; PME, phosphomonoesters.

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Oxfordshire and nearby counties. Criteria for inclusion were (*i*) a family history of gout in at least two generations and (*ii*) convincing evidence of gout in the patient including hyperuricemia, a clinical history of typical attacks of acute gouty arthritis, presence of tophi in occasional patients, demonstration of urate crystals in synovial effusions in a portion of patients, and eventual cessation of recurrent attacks with pharmacological reduction of the serum urate into the normal range.

The major criterion for exclusion from the study was the presence of a cardiac pacemaker or of other metal implanted in the body by surgery or by injury. Three of the 14 patients were eliminated because of the inability to obtain satisfactory signal-to-noise ratio in the MR spectroscopy because of obesity or unusually large body build in relation to the central cavity of the magnet used. All studies were performed after an overnight fast and at least 7 days after stopping allopurinol or other medications known to affect uric acid production or excretion and in most patients after 6 days of a diet essentially free of purines. Patients were given written instructions for following the diet containing minimal amounts of dietary purines for a 6-day period and instructed to collect urine for 24 hr on days 4, 5, and 6 of the diet; urine was collected in plastic containers with 0.2 g of thymol as preservative, thus permitting storage at room temperature to minimize the tendency for precipitation of uric acid until analysis for uric acid and creatinine. The second serum urate was taken as a single sample between 50 and 70 min after oral ingestion of fructose. Creatinine was measured in urine by a modified kinetic Jaffe method (kit 4040-7042), and urate was measured in urine and plasma by a modified phosphotungstate method (kit 4170-4172), using a Parallel analyzer obtained from American Monitor (Burgess Hill, Sussex, U.K.). The low-fructose diet consisted of avoiding all fruit, all foods containing sucrose, and those vegetables containing significant amounts of fructose (9) along with a constant diet very low in purine content with dairy products providing the major source of protein (10).

MR Spectroscopy (11). A 1.9-tesla 60-cm-bore magnet, operating at 32.7 MHz, was used for obtaining the ^{31}P MR spectra. A double-surface coil was used (12) in which the receiver coil (6.5 cm diameter) was isolated from and positioned forward of the transmitter coil (15 cm diameter). Each subject lay on his or her right side with the liver directly over the center of the coil. The field homogeneity was adjusted by observing the proton signal from tissue water. ^{31}P MR spectra of liver were obtained free of contamination from overlying muscle by using a pulse width of 180° at the surface. The interpulse delay was 1 sec. Two or three control spectra were collected for 10–15 min before the oral administration of 50 g of fructose (Frusiana, pure fructose for culinary use, marketed by Suomen Xyrofin Oy, Kotka, Finland) dissolved in 200 ml of water, and then spectra were collected at 5-min intervals for 30–40 min.

Spectra were processed by Lorentzian to Gaussian transformation after convolution difference to remove a broad underlying phospholipid peak from the spectrum. Peak heights were measured from a uniform baseline for each study and the percent change from the initial control values was plotted against time.

RESULTS

Spectral changes in response to the fructose load similar in magnitude to those observed in earlier studies of obligate heterozygotes for hereditary fructose intolerance (6) were found in 2 of the 11 patients studied with familial gout. The similarities were found not only in the time course of changes in P_i and PME peak height shown in Figs. 1 and 2 but also in the chemical shift observed in the PME peak not only in the

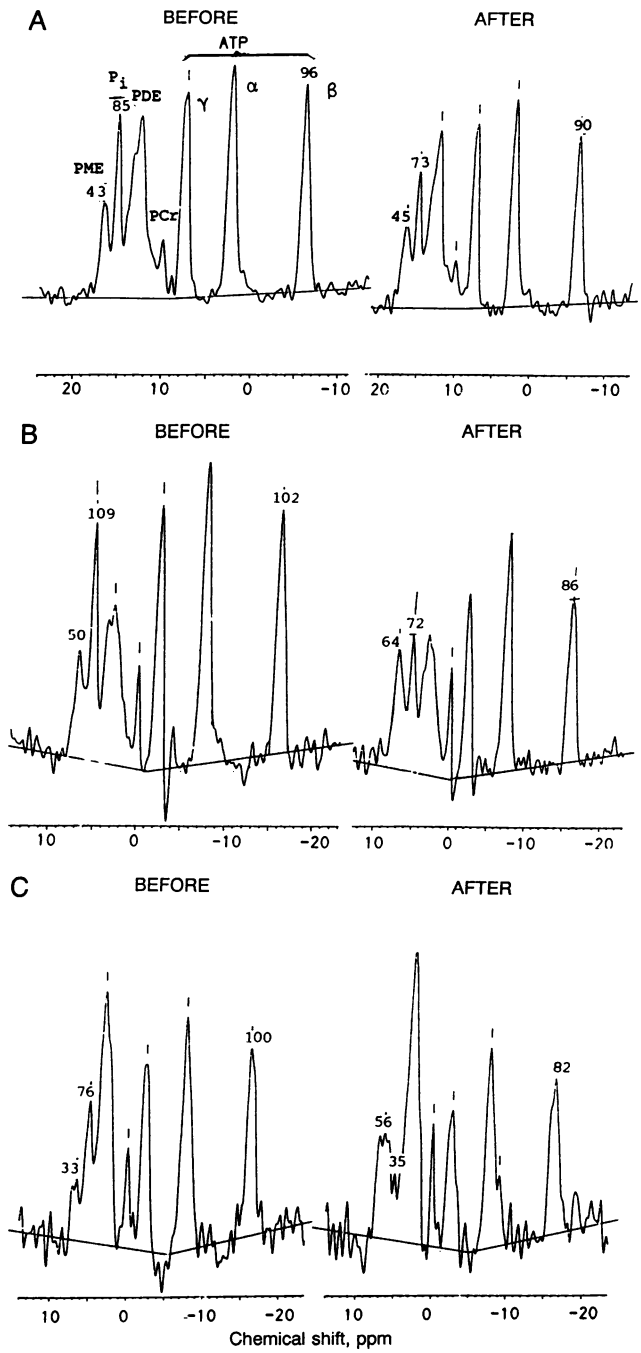

FIG. 1. Changes in ^{31}P MR spectra with time in index patients (*B* and *C*) with familial gout before and after oral administration of 50 g of fructose as compared to the nongouty nonaffected father of family 2 (control) (*A*). PDE, phosphodiester; PCr, phosphocreatinine.

index patients but also in affected family members. The PME peak shifts to lower field by an average of 0.2 ppm after fructose administration in those subjects showing an abnormal response and by 0.1 ppm in the subjects who do not show a response. This suggests that the increase in the PME peak is due to a sugar phosphate, such as fructose 1-phosphate, as such compounds resonate to a lower field than other probable components of the liver PME peak, such as phosphorylcholine.

There is considerable variation in the peak changes induced by fructose seen in heterozygotes for hereditary fructose intolerance and controls (6). Since the fructose is given orally, there is likely to be some biological variation in the rates of absorption of the fructose. As a result the cut-off points between normal and affected have been chosen quite

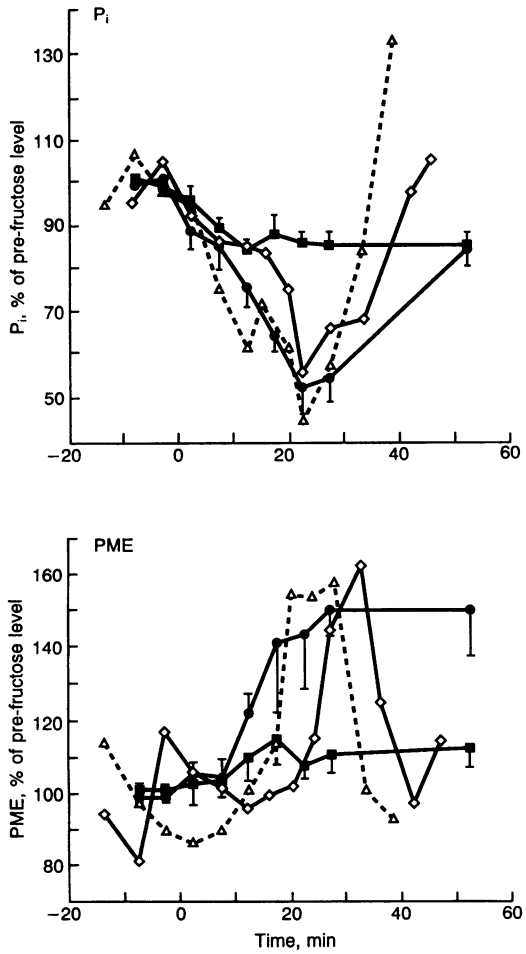

FIG. 2. Changes in P_i and PME peaks of MR spectra with time after oral administration of 50 g of fructose to index patients (◇, patient 1; △, patient 2) compared to controls (■) and obligate heterozygotes (●) of a previous study (6) with bars indicating SEM.

arbitrarily. In the current study we have, therefore, considered a response to be affected if there was a decrease of liver P_i to <70% of its initial value during the 20–30 min after administration of fructose associated with an increase in liver PME to at least 134% within the 30–40 min after fructose administration. The changes in peak heights of each of these two index patients before and after fructose administration are shown in Fig. 1 along with the values obtained from a nonaffected nongouty control subject, the father of patient 2. In Fig. 2 the time course of change in P_i and PME peaks observed in the index patients before and after the ingestion of 50 g of fructose is compared with the mean values previously obtained in this laboratory for six obligate heterozygotes for aldolase B deficiency and nine control subjects (6).

Family Studies. The relative maximal changes of the spectral peaks induced by fructose ingestion in the affected and nonaffected members of each family of the two index patients are shown in Table 1. Included for comparison is a summary of the values obtained in earlier studies of obligate heterozygotes for aldolase B deficiency and controls (6). None of the nine additional gout patients studied showed responses that were abnormal. In family 1, the index patient showed evidence of the metabolic aberration of fructose metabolism. Neither his son nor daughter was affected. The patient's father, age 85, had a history of onset of his first acute attack of gouty arthritis at age 80, confirmed by finding hyperuricemia, but neither parent was available for our study because of age and disability, so the familial correlation is presumptive in this family. In family 2, the history of gout was from the mother's side of the family, although she had a serum urate within the normal range and gave no personal history of having had gout. However, her four sons showed changes in their P_i spectra in response to the fructose load, whereas the nongouty father showed no significant change. In the five family members showing a response, P_i fell to <70% within 30 min of fructose ingestion. Three had raises in PME peaks that were clearly greater than controls. In the remaining two (mother and one son), a steady baseline PME was not achieved but the magnitude of increase in PME peak itself in each resembled that of affected members. Only one son, age 31, had clinical gout over the preceding 4 years. The oldest son, age 40, gave a history of relatively mild pain, swelling, redness, and warmth of the right first metatarsophalangeal joint 9 months earlier that persisted for 1 week. The symptoms were not incapacitating so he did not seek medical attention. Although his mother had never experienced joint symptoms, his maternal grandmother developed gout at age 50 and the grandmother's brother had onset of gout at age 60. Both then suffered from the gout for many years. In addition the same grandmother's maternal female cousin, now age 60, also has gout.

Table 1. Maximum changes in MR spectra after ingestion of 50 g of fructose

Subject	P_i, % of prefructose value	PME, % of prefructose value
Familial gout		
Family 1		
R.L. (index)	56	162
D.L. (son)	78	130
S.L. (daughter)	67	115
Family 2		
J.A.B. (index)	45	157
P.B. (brother)	66	169
A.B. (brother)	38	134
J.B. (brother)	60	115*
M.B. (mother)	64	123*
L.B. (father, unaffected)	80	114
Other gouty volunteers		
T.K. (female)	65	102
J.H.R.	73	110
T.H.	50	60
V.G.	68	103
M.W.	72	116
P.M.	70	92
T.M.	73	126
P.A.	70*	154*
J.O.	74	91
Control values	81 ± 5	115 ± 10
Obligate heterozygotes	51 ± 10	150 ± 30

Control values are from ref. 6 and are expressed as mean ± SD, $n = 9$; obligate heterozygotes are from ref. 6 and are expressed as mean ± SD, $n = 6$.

*These patients showed unsteady baselines making the peak values of questionable reliability. In addition in patient P.A. both peak values occurred together at 11 min as further evidence that these are spurious peaks.

Clinical Correlations with MR Spectral Changes After Fructose Ingestion. As shown in Table 2, only the index patient of family 1 showed an excessive production of uric acid (>600 mg/day) (10). However, on the second day of a diet essentially free of fructose as well as of purines, the 24-hr excretion of uric acid had decreased to 623 mg and the ratio of uric acid to creatinine had also decreased from 0.54 to 0.43. Although the son of patient 1 showed a mild hyperuricemia, which had been known since age 15, his production of uric acid was in

Table 2. Clinical biochemical correlates with aberrant MR spectral response to fructose

Subject	Age, years	Gout, years	Effect of 50 g of oral fructose on serum urate: Serum urate, mg/dl Before	After	Increase, mg/dl Unaffected	Affected*	Excreted in 24-hr urine, mg Uric acid	Creatinine
Family 1								
R.L. (father)	53	20	7.3	9.0		1.7	769	1508
D.L. (son)	25	0	7.0	7.6	0.6		506	1445
S.L. (daughter)	24	0	5.3	NA	—		379	1034
Family 2								
J.A.B. (son)	31	4	8.6	10.3		1.7	546	1479
P.B. (son)	40	0.7	5.3	7.0		1.7	430	1263
A.B. (son)	36	0	5.7	7.6		1.9	349	609
J.B. (son)	25	0	6.1	7.4		1.3	519	1113
M.B. (mother)	60	0	5.1	6.5		1.4	269	387
L.B. (unaffected father)	68	0	5.5	6.3	0.8		267	427
Other gouty volunteers								
T.K.† (female)	28	4	9.4	10.0	0.6		218	971
R.J.	54	2	7.1	7.7	0.6		852	2478
J.G.	67	17	8.5	8.7	0.2		470	1390
P.B.†	65	30	9.3	9.8	0.5		426	654
J.H.R.	69	20	8.9	9.5	0.6		NA	1042
T.H.	61	10	7.4	7.8	0.4		382	1066
V.G.	39	9	7.5	NA	—		463	897
M.W.§	42	3	7.0	7.2	0.2		NA	NA
P.M.	36	2	9.5	NA	—		289	1297
T.M.	57	10	8.7	9.4	0.7		NA	NA
P.A.	50	4	6.4	7.1	0.7		388	1075
J.O.	36	2	NA	8.7	—		505	1379
Mean difference ± SD					0.53 ± 0.19	1.6 ± 0.20		

NA, data not available. For patient J.G., the study was terminated at 22 min after fructose administration because of severe shoulder pain and a blood sample was taken at 30 min after the fructose. Obesity was also associated with an unsatisfactory signal-to-noise ratio in both patients J.G. and P.B. Patient R.J. was an unusually large man. A satisfactory signal-to-noise ratio could not be obtained despite attempts on 3 days.
*Patients showing aberrant response of MR spectra to ingestion of 50 g of fructose.
†Impaired renal function.
‡Hereditary nephropathy and gout.

the normal range. This suggests that the father and his 25-year-old son may not have the same basic cause of their hyperuricemia. Further evidence for this view comes from the failure of the son and the father to show the same pattern of MR spectral response to fructose (Table 1). By contrast, all of the members of family 2 produced uric acid within the normal range (Table 2). However, in both families studied each of the members carrying the defect showed a larger increment in serum urate in response to the fructose load than was shown by the nonaffected family members or any of the other gouty patients in the study as shown in Table 2. The serum urate of all of the members of family 2, with the exception of the index patient, was in the normal range after equilibration on the purine-free diet. A substantial reduction in serum urate was observed in the index patient of family 2 during restricted fructose intake. Before starting dietary treatment his level was 9.5 mg/dl. It fell to 7.6 mg/dl after 10 days of a low-fructose diet, returning to 9.4 mg/dl within 3 days of his returning to a normal diet.

DISCUSSION

Several lines of evidence support the view that heterozygosity for deficiency of fructose 1-phosphate aldolase is responsible for a significant portion of patients with familial gout. The changes observed in MR spectra and the chemical shift observed in the PME peak in response to an ingested load of fructose in 2 of the 11 patients with familial gout in the current study resembled quite closely the changes observed earlier in obligate heterozygotes identified in families with hereditary fructose intolerance (6). Earlier studies have demonstrated the ability of fructose infusion to generate a hyperuricemia in both gouty and nongouty volunteers and to increase by severalfold the rate of incorporation of [^{14}C]glycine into urinary uric acid (7, 8).

Possible Biochemical Mechanism for the Fructose-Induced Increase in Purine Metabolism. The biochemical basis for the increase in PMEs and the decrease in P_i induced by a fructose load is well grounded. There are two possible explanations. According to one group of investigators, aldolase B has a lower activity than fructokinase even in normal liver so that fructose 1-phosphate accumulates at the expense of P_i when fructose is given rapidly (13). Another group has evidence that both enzymes have the same V_{max} and the fact that the reactants of aldolase remain at equilibrium indicates that these enzymes remain highly active (14). Therefore, the accumulation of fructose 1-phosphate could result from the substantially slower metabolic pathways that convert triose phosphate into glucose and pyruvate/lactate. Regardless of just which mechanism obtains, the lower activity of aldolase B in the liver of parents of patients with hereditary fructose intolerance and of other individuals heterozygous for the adolase B deficiency would provide a reasonable basis for the greater effect of fructose ingestion on the pattern of the spectral response observed in heterozygous family members in the earlier study (6) as well as in the two patients with hereditary gout and the affected family members who showed a similar pattern in the current study. One possible mechanism for the increased sensitivity to fructose in some families with gout is certainly a decreased ability of aldolase B to split fructose 1-phosphate. The complexity of fructose metabolism, however, also provides some other possibilities.

(*i*) The velocity of the transport of fructose inside the hepatocyte could be increased. This transport is an important limiting factor in the metabolism of fructose (3).

(*ii*) The activity of triokinase could be decreased. This enzyme has a much lower activity than fructokinase and aldolase and thus plays a limiting role in the metabolism of fructose 1-phosphate.

(*iii*) The activity of one of the enzymes along the pathways that lead from the triose phosphates to glucose, on one hand, and to lactate/pyruvate on the other could be decreased. This would also slow down the metabolism of fructose 1-phosphate. Whether or not the tendency of the P_i to show a terminal overshoot and the shorter duration of decrease in P_i peaks and of elevation of PME peaks (observed in Fig. 2) than is shown in the obligate heterozygotes reflect any of the above differences remains to be determined.

Fructose-induced hyperuricemia results from a prompt degradation of liver adenine nucleotides first shown in animals (15) and later in humans (16, 17). The rapid decrease in ATP content of liver results from its participation in the rapid phosphorylation of fructose by fructokinase. A simultaneous profound depletion of P_i found in liver is reflected also in serum and is produced by its participation in the rephosphorylation of ADP in mitochondria. The degradation of AMP normally occurs in liver cells by way of AMP deaminase, which is also the rate-limiting step for catabolism of hepatic adenine nucleotides. This enzyme shows a complex system of allosteric regulators with ATP being a potent activator and P_i and GTP being inhibitors. AMP deaminase is 95% inhibited at physiological concentrations of substrates and effectors (18). The enhanced catabolic activity of adenylic acid deaminase in response to the lowered intracellular P_i and GTP results in an increased degradation of intracellular purine nucleotides and a compensatory enhancement of purine synthesis through operation of the normal feedback control mechanisms (8, 19–21). This involves conversion of phosphoribosyl pyrophosphate amidotransferase, the limiting enzyme of the *de novo* pathway of purine synthesis, from its inactive larger to its active small form (1, 19). In addition, phosphoribosyl pyrophosphate, the substrate for this enzyme, is increased manyfold (19, 22), which also increases the recovery of hypoxanthine by the salvage pathway enzyme hypoxanthine guanine phosphoribosyltransferase (1, 22). The same mechanism would also account for the greater increase in serum urate observed in all of the family members who showed the abnormal spectral response to the fructose load. This consistently larger increment in serum urate may well prove to be a much more convenient preliminary screening test for identifying affected members of the gouty population.

The fact that not all individuals carrying the defect in fructose metabolism showed a hyperuricemia provides an example of the "incomplete penetrance" frequently observed in genetics and may well reflect physiological differences in renal clearance, in residual enzyme activity, or the influence of other genes on the clinical expression of the metabolic defect. The lowering of the serum urate of an affected gouty patient to near the normal range with restriction of fructose ingestion and the lowering of the urinary uric acid to the near normal range in index patient 1 with restriction of fructose intake is further evidence of a causal role for aberrant fructose metabolism in generating the gout in these patients. It also suggests the possibility of a dietary approach to the clinical management of patients with this disorder. Earlier reports of a beneficial effect of fructose restriction in management of some, but not all, patients with gout now suggest the possibility that the patients who benefited may have been heterozygotes for aldolase B deficiency (23).

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